

UCLA iGEM 2014 – REAGENTS LIST

[The following are a list of REAGENTS that are necessary to perform the collection of recombinant silk protocols. Each section is subdivided into their respective categories, along with amounts and catalog numbers.]

Chemicals and solvents

Sterile deionized water

Tris base (Fisher Scientific Ltd, cat. no. BP152-5)

Ethylenediamine tetraacidic disodium salt dehydrate (EDTA) (Fisher Scientific Ltd, cat. no. BP120-1)

Tryptone (Fisher Scientific Ltd, cat. no. DF0118-17-0)

Yeast extract (Fisher Scientific Ltd, cat. no. DF0886-17-0)

Agar (Fisher Scientific Ltd, cat. no. DF0145-17-0)

Ampicillin sodium salt (Aldrich-Sigma Chemical Co. Ltd, cat. no. A9518; see [REAGENT SETUP PROTOCOL](#))

NaOH (Fisher Scientific Ltd, cat. no. BP 359-500)

Hydrochloric acid (HCl) (EMD, cat. no. HX 0603-4)

D-Glucose (Mallinckrodt Chemicals, cat. no. 4912-12)

GenePure LE Agarose (ISC BioExpress, cat. no. 3120-500)

Potassium acetate (Aldrich-Sigma Chemical Co. Ltd, cat. no. 236497)

IPTG (Aldrich-Sigma Chemical Co. Ltd, cat. no. I6758; see [REAGENT SETUP PROTOCOL](#))

5-Bromo-4-chloro-3-indolyl-b-D-galactopyranoside

(*X-gal*; Aldrich-Sigma Chemical Co. Ltd, cat. no. B4252; see [REAGENT SETUP PROTOCOL](#))

Agarose NA (GE Healthcare, cat. no. 17-0554-02)

NaCl (EMD, cat. no. SX0420-5)

MgCl₂ (EM Science, cat. no. MX0045-2)

Phenyl methyl sulfonyl fluoride

(*PMSF*; Aldrich-Sigma Chemical Co. Ltd, cat. no. P7626; see [REAGENT SETUP PROTOCOL](#)) **!CAUTION Flammable and toxic if inhaled.**

Deoxycholic acid (Aldrich-Sigma Chemical Co. Ltd, cat. no. D5670)

Glycine (Bio-Rad, cat. no. 161-0724)

Methanol (Fisher Scientific, cat. no. BP1105-4) **!CAUTION Flammable and toxic if inhaled.**

KCl (Aldrich-Sigma Chemical Co. Ltd, cat. no. P9541)

Na₂HPO₄ (EMD, cat. no. 8210)

KH₂PO₄ (JT Baker Chem. Co., cat. no. 53246)

Nonfat dry milk (*Carnation brand Nestle*)

Tween-20 (Aldrich-Sigma Chemical Co. Ltd, cat. no. P1379)

Sodium dodecyl sulfate (SDS; dilute to 10% (wt/vol) in water; Fisher Scientific Ltd, cat. no. BP166-500)

Bromophenol blue (dilute to 0.5% (wt/vol) in water; Aldrich-Sigma Chemical Co. Ltd, cat. no. B8026)

β-Mercaptoethanol (Aldrich-Sigma Chemical Co. Ltd, cat. no. M3148)

Bovine serum albumin (Aldrich-Sigma Chemical Co. Ltd, cat. no. A7030)

Coomassie Brilliant Blue R-250 (Aldrich-Sigma Chemical Co. Ltd, cat. no. 27816)

Glacial acetic acid (Fisher Scientific Ltd, cat. no. UN2789)

Glycerol (Shelton Scientific, cat. no. IB15762)

Nickel(II) sulfate hexahydrate (NiSO₄·6H₂O; Aldrich-Sigma Chemical Co. Ltd, cat. no. N4882-250G)

Imidazole (Alfa Aesar, cat. no. 288-32-4)

Clorox bleach (*WALMART Stores*)

Ammonium bicarbonate (Aldrich-Sigma Chemical Co. Ltd, cat. no. A141-1kg)

HFIP (TCI America, cat. no. H0424) **! CAUTION highly toxic.**

Isopropyl alcohol or 2-propanol (Aldrich-Sigma Chemical Co. Ltd, cat. no. 67 63 0)

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Vectors and bacterial strains

pBluescriptII SK(+) cloning plasmid vector (Stratagene, cat. no. 212205)

pET-19b expression vector (Novagen, cat. no. 69677-3)

***E. coli* XL1-Blue** competent cells (Stratagene, cat. no. 200249)

***E. coli* BL21(DE3)** competent cells (Stratagene, cat. no. 200132)

Enzymes and antibodies

Restriction endonucleases: *Bam*HI, *Bsp*EI, *Nde*I, *Sca*I, *Xma*I

(New England Biolabs Inc., cat. no. R0136S, R0540S, R0111S, R0122S, R0180S, respectively)

T4 DNA ligase (Promega, cat. no. M1801)

Lysozyme (Aldrich-Sigma Chemical Co. Ltd, cat. no. 62970)

DNase I (Aldrich-Sigma Chemical Co. Ltd, cat. no. D7291-5MG)

RNAse A (Aldrich-Sigma Chemical Co. Ltd, cat. no. R6513-10MG)

6 His mAb–HRP conjugate (Clontech, cat. no. 631210)

Molecular weight markers

Lambda DNA/*Hind*III marker (Promega, cat. no. G1711)

1 kbp DNA step ladder (Promega, cat. no. G6941).

Precision Plus Protein Standards Dual Color (Bio-Rad, cat. no. 161-0374)

Kits

QIAquick Gel Extraction Kit (Qiagen, cat. no. 28704)

QIAprep Spin Miniprep Kit (Qiagen, cat. no. 27104)

PureYield Plasmid Maxiprep System (Promega, cat. no. A2392)

ABI PRISM BigDye terminator Cycle Sequencing Ready Reaction Kit

(Perkin Elmer Applied Biosystems, cat. no. 4303573)

ECL western blotting detection reagents (Amersham Pharmacia Biotech, cat. no. RPN 2209)

Micro BCA Protein Assay Kit (Pierce, cat. no. Pi-23235)

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Buffers and solutions

Tris-HCl buffers (1 M (pH 8) and 0.5 M (pH 6.8); see [REAGENT SETUP PROTOCOL](#))

EDTA (0.5 M (pH 8); see [REAGENT SETUP PROTOCOL](#))

TE buffer (see [REAGENT SETUP PROTOCOL](#))

LB medium (Luria Bertani broth; see [REAGENT SETUP PROTOCOL](#))

GTE buffer (50 mM D-glucose, 25 mM Tris-HCl (pH 8), 10 mM EDTA)*

Alkaline lysis buffer (0.2 M NaOH, 1% SDS)*

RNAse A 10 mg ml⁻¹ stock*

Potassium acetate buffer (pH 4.8)*

DNAse I 2 mg ml⁻¹ stock *

Lysis buffers (1x and 8x; see [REAGENT SETUP PROTOCOL](#))

Charge buffer (see [REAGENT SETUP PROTOCOL](#))

Binding buffers (1x and 8x; see [REAGENT SETUP PROTOCOL](#))

Wash buffers (see [REAGENT SETUP PROTOCOL](#))

Elution buffers (see [REAGENT SETUP PROTOCOL](#))

Strip buffer (see [REAGENT SETUP PROTOCOL](#))

Running buffer for agarose gels electrophoresis (50x and 1x TAE buffers; see [REAGENT SETUP PROTOCOL](#))

Sample buffer (2x SDS reducing buffer; see [REAGENT SETUP PROTOCOL](#))

10x electrode buffer for SDS-PAGE (10x EB; see [REAGENT SETUP PROTOCOL](#))

10x phosphate-buffered saline (PBS) buffer (see [REAGENT SETUP PROTOCOL](#))

1x PBS-Tween (PBST) buffer (see [REAGENT SETUP PROTOCOL](#))

Blocking buffer (see [REAGENT SETUP PROTOCOL](#))

10x NEB Buffer 2

10x NEB Buffer 3

5x Ligase Reaction Buffer

*** Refer to**

Sambrook, J. & Russell, D.W. (eds.) Molecular Cloning: A Laboratory Manual, 3rd edn., Vol. 1–3 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA, 2001).

For methods on preparation and storage.

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Reagent Setup Protocol

1 M Tris-HCl (pH 8):

For 1 liter, dissolve 121.1 g of Tris base in 800 ml of deionized water. Adjust the pH to 8 with HCl (about 42 ml) and add water to make up a final volume of 1 liter of water. Store at room temperature (20–24°C) for up to 1 year.

0.5 M Tris-HCl (pH 6.8):

Dissolve 60.55 g of Tris base in 800 ml of deionized water. Adjust the pH to 6.8 with HCl and add water to make up a final volume of 1 liter. Autoclave Tris-HCl buffers to sterilize. Alternatively, purchase 0.5 M Tris-HCl (pH 6.8; Bio-Rad, cat. no. 161-0799). Store at 4°C for up to 1 year.

0.5 M EDTA (pH 8):

Dissolve 186.1 g of EDTA in 800 ml of deionized water. Adjust the pH to 8 with NaOH solid pellets (about 20 pellets). Autoclave to sterilize. Store for at 4°C for up to 1 year.

TE buffer (10 mM Tris-HCl, 1 mM EDTA (pH 8)):

For 100 ml, add 1 ml of 1 M Tris-HCl (pH 8), 0.2 ml of 0.5 M EDTA (pH 8) and water to make up a final volume of 100 ml. Autoclave to sterilize. Store at room temperature for up to 1 year.

Ampicillin 50 mg ml stock:

Dissolve 50 mg of ampicillin in 1 ml of deionized water. Filter-sterilize using a 0.22-µm-pore-size filter, aliquot and store at -20°C for up to 1 year. Use at a final concentration of 50 µg ml⁻¹ in liquid LB or LB agar plates.

IPTG 100 mM stock:

Dissolve 0.238 g in 10 ml of deionized water. Filter-sterilize using a 0.22-µm-pore-size filter. Aliquot and store at -20°C for up to 2 years. Use at a concentration of 0.5 mM.

X-gal 20 mg ml⁻¹ stock:

Dissolve 20 mg of X-gal in 1 ml of *N,N'*-dimethylformamide. Protect from light by wrapping the stock in aluminum foil, aliquot and store at -20°C for up to 1 year. Use at a final concentration of 80 µg ml⁻¹.

PMSF 100 mM stock:

Dissolve 0.84 g of PMSF in 50 ml of 100% ethanol. Vortex solution and store at -20°C for up to 1 year.

!CAUTION PMSF is harmful to health when inhaled or in contact with the skin and can cause irritation and sensitization.

LB medium:

Dissolve 10 g of tryptone, 5 g of yeast extract, 10 g of NaCl in 900 ml of deionized water. Add water to make up a final volume of 1 liter. Autoclave to sterilize. When required, add antibiotics (50 µg ml⁻¹ ampicillin) to the sterile media after cooling to 50°C. Store at 4°C for up to 2 weeks.

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LB agar plates:

Add 15 g of agar to 1 liter of LB medium (without mixing). Autoclave to sterilize. When required, add antibiotics (50 $\mu\text{g ml}^{-1}$ ampicillin) to the sterile media after cooling to 50°C and store at 4°C for up to 2 weeks.

CRITICAL: To prepare LB agar plates for blue-white colony screening, add X-gal and IPTG to a final concentration of 80 $\mu\text{g ml}^{-1}$ and 0.5 mM, respectively, when adding the antibiotic.

8x lysis buffer:

Prepare a buffer containing 400 mM Tris-HCl (pH 8), 80 mM MgCl_2 , 80 mM NaCl. Store at 4°C for up to 2 months. Use at a 1x concentration, i.e., 50 mM Tris-HCl (pH 8), 10 mM MgCl_2 , 10mM NaCl, for IMAC.

Charge buffer:

50 mM NiSO_4 prepared in water. Prepare fresh every time.

8x binding buffer:

Prepare a buffer containing 40 mM imidazole, 4 M NaCl, 160 mM Tris-HCl (pH 8). Store at 4°C for up to 2 weeks. Use at a 1x concentration, i.e., 5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl (pH 8) for IMAC. The 8x binding buffer may also serve as a stock to prepare the different 1x wash buffers (with imidazole concentration of 40 and 50 mM) and elution buffers (with imidazole concentrations equal or superior to 80 mM).

Elution buffer:

80 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl (pH 8). Prepare an 8x elution buffer stock (640 mM imidazole, 4 M NaCl, 160 mM Tris-HCl (pH 8)) and adjust the imidazole concentrations to 100 or 250 mM when preparing the 1x elution buffers to be used for IMAC. Store at 4°C for up to 2 weeks.

Strip buffer:

100 mM EDTA, 0.5 M NaCl and 20 mM Tris-HCl (pH 8). Store at 4°C for up to 1 year.

Running buffer (1x TAE buffer: 40 mM Tris acetate, 1 mM EDTA (pH 8)):

Make a 50x TAE stock by mixing 242 g of Tris base with 57.1 ml of glacial acetic acid, add 100 ml of 0.5 M EDTA (pH 8) and adjust the volume to 1 liter. Store at room temperature for up to 1 year. Dilute the 50x TAE to 1x for electrophoresis.

Sample buffer (2x SDS reducing buffer):

For 10 ml, mix 3.55 ml of water, 1.25 ml of 0.5 M Tris-HCl (pH 6.8), 2.5 ml of glycerol, 2 ml of 10% (wt/vol) SDS and 0.2 ml of 0.5% (wt/vol) bromophenol blue. Store at 4°C for up to 1 year. Add 50ml of β -mercaptoethanol to 950 μl of sample buffer before use.

!CAUTION β -Mercaptoetanol is highly toxic. SDS is also harmful to health when inhaled or in contact with the skin, and can cause irritation and sensitization.

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10x EB (pH 8.3):

For 1 liter, dissolve 30.3 g of Tris base, 144 g of glycine and 10 g of SDS in deionized water to make up a final volume of 1 liter. Store at 4°C for up to 1 year. If precipitation occurs, warm the buffer to room temperature before use. Alternatively, purchase the 10x Tris/glycine/SDS electrophoresis running buffer (Bio-Rad, cat. no. 161-0772) and use at a 1x concentration for electrophoresis.

10x PBS (1.37 M NaCl, 27 mM KCl, 100 mM Na₂PO₄, 18mM KH₂PO₄):

For 1 liter, dissolve 80.6 g of NaCl, 2.013 g of KCl, 14.196 g of Na₂PO₄ and 2.449 g of KH₂PO₄ in 850 ml of water. Adjust the volume to 1 liter once the salts are dissolved. Store at 4°C for up to 1 year.

1x PBST (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂PO₄, 1.8mM KH₂PO₄, 0.05% (vol/vol) Tween-20):

For 1 liter, mix 100 ml of 10x PBS, 0.5 ml of Tween-20 and 899.5 ml of water. Prepare fresh every time.

Blocking buffer 1x PBST/5% nonfat dry milk:

For 100 ml of blocking buffer, mix 100 ml of 1x PBST with 5g of nonfat dry milk. Prepare fresh every time.

90% isopropyl alcohol coagulation bath:

For 1 liter, add 100 ml of distilled water to 900 ml of isopropyl alcohol. Prepare fresh every time.

!CAUTION Isopropyl alcohol is highly flammable.

This protocol is adapted from the following source:

Teule, F., Cooper, A.R., Furin, W.A., Bittencourt, D., Rech, E.L., Brooks, A., & Lewis, R.V. "A protocol for the production of recombinant spider silk-like proteins for artificial fiber spinning." *Nature Protocols*, Vol. 4, No. 3, 19 February 2009 (online). Pages 341-355. Nature Publishing Group. doi:10.1038/nprot.2008.250